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Evaluation of the rodenticidal effects of some plant extracts under laboratory and field conditions

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Abstract The rodenticidal effects of seven crude plant extracts (Calendula, sumac, Damsissa, lemon grass, worm wood, Duranta and camphor) solved by ethanol, hexane and petroleum ether were studied under laboratory and field conditions at the Menoufia Governorate. The obtained results revealed that under laboratory conditions only three plant extracts, particularly those extracted by ethanol, promised to be used as a rodenticide against the albino rat, *Rattus norvegicus*. These are calendula, sumac and camphor extracts. Moreover, ethanolic calendula exhibited a high toxic effect against the albino rat when compared with the other two ethanolic plant extracts. The changes in the internal organs related to calendula, camphor and sumac treatments (ethanol extract) were a significant reduction in body weight, significant increases in the internal organs weight (liver, kidney, heart, brain, spleen and lung), cloudy appearance, congestion and bleeding in the liver, kidney and heart, noticeable dark color of the spleen as well as obvious congestion and bleeding in the brain.

Under the field conditions, ethanolic extract of calendula achieved the highest value of population reduction (67.7) of the Norway Rat, *R. norvegicus*. The assessment of the phytochemical constituents of the same three plant extracts indicated that glycosides, flavonoides, saponins, tannins, triterpenes and sterols may be responsible for the rodenticide effect observed in the present study.

Therefore, calendula and sumac ethanol extracts could be used as rodenticides under laboratory and field conditions.

Introduction

In recent years, the emphasis on plant protection has definitely shifted from the dominant chemical pesticides to the integrated pest management (IPM), the focus is on biological control and other natural resources with reduced reliance on chemicals (Schmutterer, 1981). Many investigators suggested that the basic research must be directed to the discovery of new safe types of pest control agents in order to ensure high production and preservation of agricultural products (Schmutterer, 1981;

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Saleh et al., 1986; Qureshi et al., 1991; Afifi et al., 1992; Oji et al., 1994).

Plant extracts have been used as pesticides by humans since before the time of the ancient romans, a practice that continues to the present time with many of the plant species known to have pesticidal properties (Fellows, 1979). The use of toxic plants is especially prevalent in the developing countries, where plants grown locally are cheaper than the synthetic chemical pesticides (El-Gengaihi et al., 1997).

Therefore, many plants, trees, shrubs, annuals, either wild or cultivated were surveyed for their pesticidal activity. Many of them have been proved to be active. These plants were phytochemically investigated to determine their chemical compositions (Hussein, 1991).

The steroidal glycosides, cardenolides, are considered the most important of all the naturally occurring products. These groups have long been used as arrow poisons or drugs (Hussein, 1991).

Muley et al. (2009) recorded many bioactive compounds in *Calendula officinalis* ethanol extract i.e. terpenoids, flavonoids, quinones, volatile oil, carotenoids, amino acids and carbohydrates.

Sanja Matic et al. (2011) revealed that the methanol extract of *Cotinus coggygria* plant stems is capable of giving rise to genotoxic effects. The partial chemical analysis of the methanol extract of *C. coggygria* showed the presence of flavonoids, tannins and phenolic compounds (Stanic et al., 2009).

The present work aims to study the rodenticidal effect of some crude plant extracts, using different extraction solvents, on albino rats under laboratory and field conditions.

Materials and methods

Tested plants

Seven plant species were assessed in the present study. Their English and Latin names, the used part of the plants and the location from which they are gathered are illustrated in Table 1.

Preparation of crude extracts

Green plant materials were washed with water and then left to dry under room conditions. The dry plant materials were ground in a grinder to a coarse powder. Extraction was carried out according to the method adopted by Freedman et al. (1979) with slight modifications where ground flowers, leaves and peels and trunks were soaked in the chosen solvents (ethanol 95%, hexane and petroleum ether) instead of using the soxhlet procedure. An amount (150 g) of each plant powder was soaked in 750 ml of each solvent for 3 days in brown colored bottles provided with light stoppers. The bottles were intermittently shaken by an electric shaker for 2 h daily. Each extract was then filtered over sodium anhydrous sulphate and evaporated to dryness under reduced pressure by a rotary evaporator at 40–50 °C in a water bath. The crude extracts were weighed and kept in a deep freezer until use. The marc was subsequently subjected to extraction with another solvent (hexane and petroleum ether, respectively) and the same procedure was used. Each crude extract was mixed with crushed maize (10 ml/kg).

Laboratory tests

Handling of animals

Adult male and female albino rats (*Rattus norvegicus*) were used for laboratory experiments. Animals were caged individually and provided with standard diet (65% crushed maize + 25% ground wheat + 5% sugar + 5% corn oil) daily for two weeks. Water was supplied ad libitum. The unhealthy and pregnant animals were excluded. Animals were weighed and each was given a reference number. Rats were then subjected to one of the following tests:

Non-choice feeding method. Groups of 10 albino rats were singly caged and each group was treated with one of the plant extracts. Each rat was daily offered 50 g crushed maize coated with one of the tested plant extracts for 4 successive days. The consumed amount of diet was calculated daily. The treated crushed maize bait was then removed. Survived individuals were kept on standard diet until the end of the experiment (28 days). During this period the mortality rate was recorded. Dead animals were autopsied to investigate the symptoms of plant extracts poisoning.

Free choice feeding method. The three plant extracts proved to cause the highest mortality rate in the non-choice feeding method were tested using the free choice feeding method. The palatability of a tested material is normally determined in the laboratory according to Palmateer (1974) by comparing its consumption with that of the challenge diet (65% crushed maize, 25% ground wheat, 5% sugar and 5% corn oil). Ten animals were used for each extract treatment. The free choice method of Buckle and Smith (1994) was as follows: 50 g from each crushed maize treated with the candidate plant extract (10 ml from each crude extract was mixed with 1 kg of crushed maize) and standard diet were offered to each rat in separate small dishes with free water. The positions of the two dishes were reversed daily to avoid preference for a certain location.

Table 1 Names, used parts and location of the different plant species studied.

English name	Latin name	Used part	Location
Calendula	*Calendula aegyptiaca*	Flowers	Giza
Damsissa	*Ambrosia maritime*	Whole plant	Giza
Worm wood	*Artemisia herba-alba Asso*	Whole plant	Giza
Duranta	*Duranta plumeria*	Leaves	Giza
Sumac	*Rhus continues*	Leaves	Giza
Lemon grass	*Cymbopogon cilratus*	Leavea	Giza
Camphor	*Eucalyptus globulus*	Peels and trunk	Giza

The consumption of treated and untreated bait was recorded daily for 4 successive days, then the treated baits were removed and survived animals were fed for 28 additional days on challenge diet. A parallel control test was conducted using each solvent standard diet. Bait acceptance and mortality percentage were recorded using the equation of Buckle and Smith (1994):

$$\text{Acceptance}\% = \frac{\text{Consumed amount of treated bait}}{\text{Consumed amount of treated bait} + \text{challenge diet}} \times 100$$

Field experiments. The three tested plant extracts, mentioned before (calendula, sumac and camphor) were evaluated under the field conditions during the year 2011 at EL-Remaly Village, Quossna District, Menoufia Governorate, according to the method described by Dubock (1982). The trials were conducted during June and July after the winter crops harvest against the Norway rat, *R. norvegicus* in field crops as an infested area, one feddan plot was a located each plant extract. In addition to one feddan left without treatment as a check control area. The population density of the rodent species was estimated pre and post treatment using the crushed maize consumption method.

A known weight of crushed maize was divided and distributed inside 20 cement bait stations (12 cm diameter and 50 cm length) in every plot for 4 successive days. Food consumption was calculated pre and post treatment. The average consumption in the last two days was estimated. Hundred grams of the plant extract bait was exposed to rats inside the bait stations and the consumed quantity of the plant extract bait was replaced every week until the stop of consumption, with recording of the consumption. The poisoned bait and bait stations were removed at the end of the treatment periods. The reduction in rodent population was estimated as follows:

$$\text{Population reduction}\% = \frac{\text{Pre-treatment consumed} - \text{post-treatment consumed}}{\text{Pre-treatment consumed}} \times 100$$

Phytochemical examination

Preliminary phytochemical constituents in the studied bioactive plant extracts. The tested plant species in the previous experiments extracted by ethanol which exhibited reasonable rodenticidal effect were selected to investigate their phytochemical constituents. These plant species were Calendula, Camphor and Sumac. Extraction was conducted by the following methods:

1. *Sterols and tritrepenes:* Sterols and triterpenes were detected according to the method described by Wall et al. (1964).
2. *Tannis:* Tannis was identified by the method described by Clause (1961).
3. *Anthraquinone:* Anthraquinone was calculated according to Balbaa (1981).
4. *Cardiac* glycosides: Cardiac glycosides were estimated according to Balbaa (1981).
5. *Alkaloids:* Alkaloids were estimated by the method described by Romo (1966).
6. *Flavonoids:* Flavonoids were determined according to the method adapted by Clause (1961).
7. *Carbohydrates and glycosides:* Carbohydrates and glycosides were determined by the method adapted by Karawya and ELWahab (1975).
8. *Saponin glycosides:* Saponin glycosides were calculated according to the method mentioned by Wall et al. (1964).

Statistical analysis

The obtained results were statistically analyzed by one way ANOVA and Least Significant Difference (LSD) at ($P < 0.05$) using Costat program (Cohort Software, 2005).

Results and discussion

Laboratory studies

Laboratory and field experiments have been conducted to evaluate the rodenticidal effects of some plant extracts on albino rats, under laboratory conditions. The rodenticidal effects of the seven plant extracts solved by three different organic solvents, were tested.

The non-choice feeding method

The obtained data revealed that among the seven plant extracts tested only three promised to have a rodenticidal potential against this rodent species (Table 2). The three extracts of Calendula, Camphor and Sumac when extracted by ethanol gave mortality rates of 100%, 80% and 90%, respectively, with an average bait consumption of 10.5, 10.2 and 11.2 g, followed by (80%, 50% and 60%) and (20%, 10% and 20%) mortality with hexane and petroleum, respectively. The lowest rate mortality percentage was extracted only in case of plant extracted with petroleum ether.

However, whole damsissa plant, lemon grass leaves, whole worm wood plant and duranta leaves did not achieve any animal mortality when extracted by ethanol, hexane or petroleum ether.

Free choice feeding method

The effects of the three plant extracts which exhibited a noticeable rodenticidal potential, on the mortality percentages of albino rats were studied using the free choice feeding method. The data obtained are summarized in Table 3. The compiled data run in parallel with those obtained from the non-choice feeding method. Ethanol Calendula extract induced complete mortality (100%) followed by 90% and 80% mortality rates for Sumac and Camphor, respectively. The mean time required for death was 6.5 days for Calendula ethanol extract and 6.6 and 6.9 days for Sumac and Camphor, respectively. On the other hand, the palatability of baits treated with the ethanol plant extracts could be arranged according to their acceptance by rats in a descending order as follows: Calendula > Sumac > Camphor.

Reviewing the aforementioned results it is obvious that ethanol extracts of the Calendula and Sumac plants proved to be the most effective when extracted by different solvents and differed according to variation in bioactive compound types. Hussein (1991), Khidr (2001) and Gabr et al., (2004) recorded many bioactive compounds in Oshar leaves and Camphor leaves and seeds extracted with ethanol such as steroids triterpenes, phenolic glycosides, tannins, anthraquinones, glycosides, flavonoids, alkaloids and carbohydrates. Ibrahim (2001) found that the ethanolic oshar leaves extract was the

Table 2 Effect of some crude plant extracts (10 ml/kg bait) against the albino rat for 4 successive days using the non-choice feeding method.

Plant	Solvent	Average bait consumption (g)	% Mortality	Time to death (days)	
				Range	Mean
Calendula	Ethanol	10.5	100	4–10	7.0
	Hexane	9.2	80	6–17	11.5
	Petroleum ether	8.5	20	9–22	15.5
Camphor	Ethanol	10.2	80	4–11	7.5
	Hexane	8.9	50	6–19	12.5
	Petroleum ether	7.8	10	20.0	20.0
Duranta	Ethanol	5.23	0.0	0.0	0.0
	Hexane	2.61	0.0	0.0	0.0
	Petroleum ether	7.2	0.0	0.0	0.0
Worm wood	Ethanol	4.3	0.0	0.0	0.0
	Hexane	4.35	0.0	0.0	0.0
	Petroleum ether	5.76	0.0	0.0	0.0
Demsissa	Ethanol	2.82	0.0	0.0	0.0
	Hexane	1.52	0.0	0.0	0.00.0
	Petroleum ether	1.55	0.0	0.0	
Sumac leaves	Ethanol	11.2	90	5–12	8.5
	Hexane	9.7	60	7–18	12.5
	Petroleum ether	8.2	20	9–21	15.0
Lemon grass	Ethanol	9.23	0.0	0.0	0.0
	Hexane	8.6	0.0	0.0	0.0
	Petroleum ether	9.19	0.0	0.0	0.0
Control	Standard diet and solvent	19.8	0.0	0.0	0.0

Table 3 Effect of some crude plant extracts (10 ml/kg bait) against the albino rat for four successive days using the free-choice feeding method.

Plant	Solvent	% Acceptance	% Mortality	Time to death (days)	
				Range	Mean
Calendula	Ethanol	55.7	100	4–11	6.5
	Hexane	48.0	70	7–14	7.3
	Petroleum ether	33.0	30	8–20	14.0
Camphor	Ethanol	53.0	80	4–12	6.9
	Hexane	44.0	40	7–17	7.9
	Petroleum ether	27.3	20	9–22	16.0
Sumac	Ethanol	55.2	90	5–13	6.6
	Hexane	46.0	50	7–15	8.2
	Petroleum ether	30.0	20	9–23	18.1
Control	Standard diet and solvent	100	0.0	0.0	0.0

most effective one followed by datura extract while black pepper seed extract exhibited the lowest toxic effect against rat.

Changes in different organs

The clinical symptoms and pathological changes in the body weight and internal organs of albino rats following treatment with Calendula, Camphor and Sumac plant ethanol extracts are presented in Table 4. The data show that body weight of animals treated with calendula and sumac ethanol extract was significantly decreased in comparison to that of untreated animals. The loss in body weight of rats post-treatment may be due to loss of appetite. In contrast, a significant increase occurred in the weight of liver, kidney, heart, brain, spleen and lung following calendula and sumac ethanol extract treatment. A noticeable darkness of color was observed in the spleen. In addition, obvious congestion and bleeding was noticed in the lung. Cloudy swelling occurred in the kidney compared to that of untreated animals. Sebaii (1996) and Faye (1985) found that oshar and other plant extracts caused internal bleeding and retardation of embryos development in pregnant albino rats. Rezk (2006) noticed that 30 mg/kg b.w. of ethanolic Oshar extract caused congestion, hemorrhage, swelling and darkness in color in the internal organs (liver, kidney and intestine) of albino rat when compared with control animals.

Field studies

Field trials have been conducted to study the rodenticidal effects of three ethanolic plant extracts against the Norway rat *R. norigicus* under the field conditions of Menoufia Governorate. Results in Table 5 indicate that Calendula ethanol extract was the most effective one as it caused 67.7% rat population followed by 64.8% and 42.7% for Sumac and Camphor plant extracts, respectively. At the same time, consumption of the

Table 4 Effect of Calendula and Sumac ethanolic extracts on different organs of albino rat.

Plant extract	Organ	Average weight (g)		Clinical symptoms
		Control Mean ± SE	Treated Mean ± SE	
Calendula	Body	190.0 ± 1.1	130.0 ± 3.4**	Reduced body weight
	Liver	3.75 ± 0.06	7.75 ± 0.36**	Cloudy appearance and congestion
	Kidney	1.23 ± 0.07	2.23 ± 0.19*	Congestion and bleeding
	Heart	0.81 ± 0.03	1.81 ± 0.09**	Cloudy appearance and congestion
	Brain	1.38 ± 0.04	1.48 ± 0.01*	Congestion and bleeding
	Spleen	0.3 ± 0.02	0.65 ± 0.07*	Dark colour
	Lung	1.26 ± 0.29	2.46 ± 0.21*	Bleeding congestion
Camphor	Body	180.0 ± 1.1	165.0 ± 3.4**	Reduced body weight
	Liver	4.1 ± 0.05	6.9 ± 0.026*	Cloudy appearance and congestion
	Kidney	1.7 ± 0.04	2.58 ± 0.16*	Congestion and bleeding
	Heart	098 ± 0.01	1.30 ± 0.05*	Cloudy appearance and congestion
	Brain	1.28 ± 0.06	1.62 ± 0.01*	Congestion and bleeding
	Spleen	0.28 ± 0.04	0.73 ± 0.09*	Dark colour
	Lung	1.19 ± 0.3	1.94 ± 0.22*	Bleeding congestion
Sumac	Body	230 ± 3.6	190.0 ± 3.7	Reduced body weight
	Liver	5.47 ± 4.6	6.23 ± 2.8	Congestion and bleeding
	Kidney	1.4 ± 3.4	2.42 ± 1.4	Cloudy appearance and congestion
	Heart	0.9 ± 1.1	1.8 ± 0.85	Congestion and bleeding
	Brain	1.3 ± 1.2	1.72 ± 1.1	Dark colour
	Spleen	0.2 ± 0.1	0.54 ± 0.1*	
	Lung	1.03 ± 0.3	2.25 ± 0.11*	Bleeding and congestion
Control	Body	175 ± 6.3	176 ± 7.3	No changes in different organs
	Liver	3.6 ± 0.3	3.61 ± 0.34	
	Kidney	1.3 ± 0.63	1.32 ± 0.45	
	Heart	0.9 ± 0.1	0.91 ± 0.02	
	Brain	0.25 ± 0.1	0.25 ± 0.12	
	Spleen	1.09 ± 0.06	1.08 ± 0.04	
	Lung	12.34 ± 0.2	12.33 ± 0.27	

* Significant ($P < 0.05$).
** Highly significant ($P < 0.01$).

poisoned bait per feddan was considerably different according to the plant extract .The consumed amounts per feddan were 1150, 1030 and 1060 g when calendula, sumac and camphor extracts were applied, respectively.

Statistical analysis revealed that the data are always significant between the three plant extracts.

Discussing the aforementioned results, it could be concluded that the toxic effect was different according to the type of the plant and the part used. Gabr et al. (2004) recorded that ethanol extract of oshar leaves induced a markedly reduced population (up to 66.7%) at Sharkia Governorate. Ibrahim (2001) recorded that ethanol oshar, datura leaves and black pepper induced a markedly reduced rat population (85.3%, 84.3% and 80.8%, respectively), at the Menofia Governorate. Mohamed (1995) and Sebaii (1996) indicated that powdered latex baited with crushed maize with different ratios proved to have strong lethal effect against rats.

From the obtained results calendula, camphor and sumac ethanol extract could be considered potential rodenticides in rodent management programs.

Preliminary screening of phytochemical constituents of studied plants

The use of botanical pesticides depends on knowing their chemical constituents, thus, it is important to undergo preliminary phytochemical screening for these plants. Three of

Table 5 Field performance of some crude plant extract against the Norway rat, *Rattus norvegicus* in field crops at El-Menofia Governorate.

Plant	Solvent	Consumption (g)/feddan			% Population reduction
		Pre-treatment	Poison bait	Post-treatment	
Calendula	Ethanol	1300	1150	380	67.7
Sumac	Ethanol	1250	1030	440	64.8
Camphor	Ethanol	1500	1060	860	42.7
control	Crushed maize	1340		1340	0.0
F test	–				Significant
LSD at 0.05					2.98

Table 6 Preliminary phytochemical screening of the tested plants extracted with ethanol.

Constituents	Ethanolic plant extracts		
	Calendula	Camphor	Sumac
Cardiac glycoside	+	+ + +	±
Flavonoids	+ + +	+ + +	+ + + +
Saponin glycosides	±	+ +	+
Sterols and triterpenes	+	+ +	+
Carbohydrates and glycosides	+ + +	+ + +	+ + +
Alkaloids	+	+ +	+
Anthraquinone	+	+ +	+
Tannins	+ + + +	+ + +	+ +

+ + + + very high amount, + + + high amount, + + moderate amount, + slight amount, ± trace amount.

the tested plant species in the present study exhibited a high toxic effect against the albino rat, *R. norvegicus*, and were selected to investigate their phytochemical constituents. These plant species were Calendula, Sumac and Camphor, but in various amounts due to plant part and solvent of extraction. The dried powder of the tested plants was subjected to this screening for the detection of sterols and triterpenes, tannins, anthraquinone glycosides, saponin, flavononids, cardiac glycoside, alkaloids and carbohydrates and glycosides. The obtained results are presented in Table 6. Data indicated that the following constituents are detected in the ethanol extract of the tested plants:

1. Flavonoids, carbohydrates and glycosides and tannins were found in high amount in the tested plant extracts.
2. Sterols and triterpenes, anthraquinone and glycoside and alkaloids were found in moderate amounts in Camphor extracts with ethanol while found in slight amounts in Sumac and Calendula ethanolic extract.From the obtained results it could be concluded that sterols and triterpenes, anthraquinone, flavonoids, alkaloids and carbohydrates and glycoside were found in varying amounts in all tested plants. These compounds mostly act as pesticide agents (Su, 1984, 1990). Such results are in agreement with many investigators,Edward et al. (1993) studied the terpenoid composition of 6 species of Eucalyptus, all 6 Eucalyptus species showed that cineole (eucalyptol) content ranged from 13% to 78% of the total oil.

Aly (1999) separated nine phytochemical components in leaves and seeds of both red and spotted gum. Such components were found in different amounts according to plant species, plant part and solvent used.

The partial chemical analysis of methanol extract of *C. coggygria*, showed flavonoids, tannins and phenolic compounds (Stanic et al., 2009). Muley et al. (2009) found the presence of active substances in the *C. officinalis* flowers by phytochemical study. The results obtained were terpenoids, flavonoids, quinones, volatile oil, carotenoids, amino acids and carbohydrates.

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